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GENERATION OF TARGETED C_2-SYMMETRICAL COMPOUND LIBRARIES BY SOLUTION-PHASE COMBINATORIAL CHEMISTRY

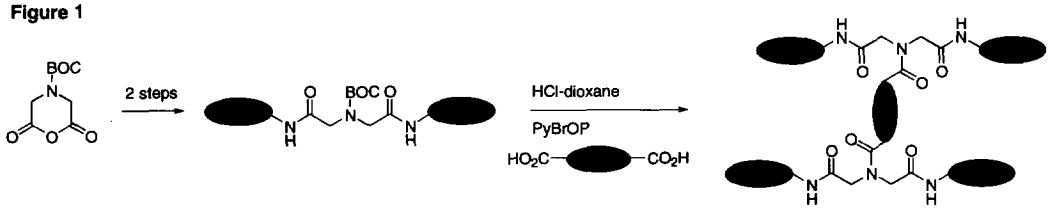
Dale L. Boger,* Rachel S. Ozer, and Carl-Magnus Andersson

Department of Chemistry, The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, California 92037

Abstract. An approach to the preparation of C_2-symmetrical chemical libraries for use in protein and receptor homodimerization studies by solution-phase methods which permits the multi-milligram synthesis of each member is described. © 1997 Elsevier Science Ltd.

Ligand-induced receptor and protein dimerization or oligomerization has emerged as a general mechanism for signal transduction.[1] Members of several receptor families including protein tyrosine kinase receptors (homo- or heterodimerization),[2] cytokine receptors (homo- or heterodimerization),[3-6] serine/threonine kinase receptors (hetero-oligomerization),[7] and members of the TNF-receptor family (trimerization)[8] have been established to utilize this mode of activation. Similarly, intracellular signal transduction often proceeds by ligand-induced protein–protein homo- or heterodimerization.[9,10] Herein we report an effective protocol for generating C_2-symmetrical or unsymmetrical chemical libraries suitable for probing receptor and protein homo- and hetero-dimerization events (Figure 1). The approach constitutes the dimerization linkage of iminodiacetic acid diamides with a mixture of rigid dicarboxylic acids. The reaction sequence illustrated with the assembly of a 600 member library requires 3 steps and represents an extension of our solution-phase parallel synthesis of chemical libraries.[11] In addition to the advantages originally outlined,[11] the solution-phase synthesis of the intermediates permits their final direct linkage which would be precluded by solid-phase synthesis techniques. As such, the strategy is uniquely suited for taking advantage of such symmetrical dimerization utilizing a limited number of synthetic steps.[12]

Figure 1



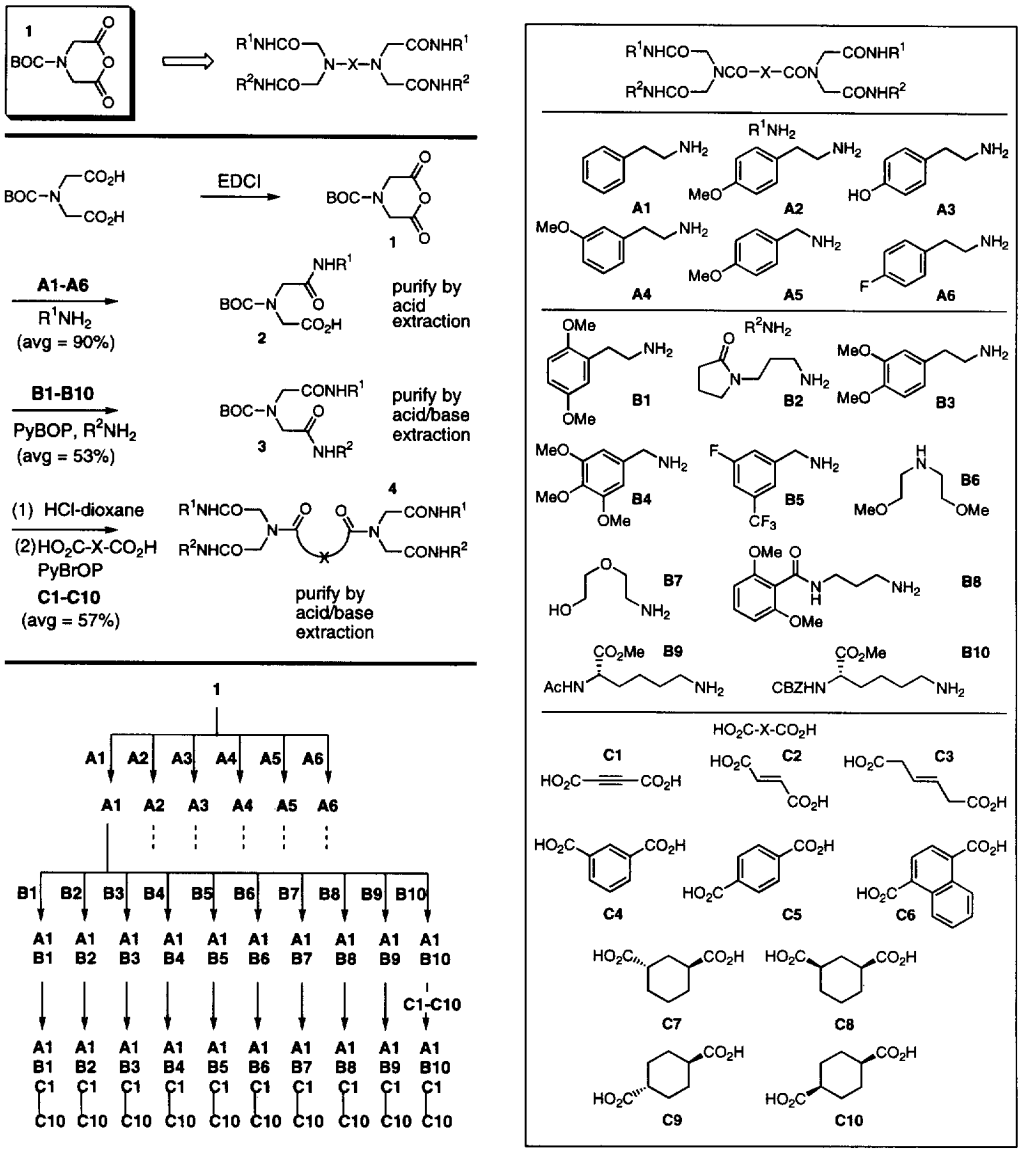
An approach that minimizes the number of chemical reactions, that maximizes the diversity impact and that provides a convenient screening/deconvolution format was devised for the preparation of a

prototypical library of 600 symmetrical compounds. This library was constructed in a 6 × 10 × 10 matrix with the final diversification being accomplished in one reaction to provide a mixture of 10 compounds containing variations in only the linking domain (Figure 2). For the 3-step synthesis, this required 6 amines (R^1NH_2, **A1–A6**), 10 amines (R^2NH_2, **B1–B10**), and 10 dicarboxylic acids (HO_2C-X-CO_2H, **C1–C10**), and the conduct of 126 reactions to produce 60 sublibraries each containing 10 compounds each. Deconvolution by resynthesis of the individual components is straightforward and the screening assays applied to a modest mixture of 10 compounds was judged optimal.[13] In addition to the advantages associated with the testing of a modest mixture of 10 compounds, the variability within each sublibrary only in the nature of the linker. This was expected to minimize false positive or negative screening results that accompany the testing of mixtures of compounds[14] and, at the same time, permits the simultaneous examination of a range of linkers. More importantly, the concurrent examination of the intermediate iminodiacetic acid diamides for antagonist versus agonist activity permits, in principle, the identification or confirmation of binders that upon linkage dimerization might function as agonists.[10] In addition, when this format is applied in a pair-wise fashion for the construction of symmetric and unsymmetric compound libraries (i.e., **A1B1 + A1B2 + C1–C10**), the final mixtures contain 30 compounds composed of each of the two symmetrical sub-libraries (10 + 10, **A1B1C1–10 + A1B2C1–C10**) plus the unsymmetrical combination (10). Testing of the modest mixture of 30 compounds constitutes the upper limit judged valuable,[13] and the full library size including the unsymmetrical combinations would be 18,300 compounds built with only 26 diversity units (6 + 10 + 10).

Reaction of *N*-BOC-iminodiacetic acid with the water-soluble coupling reagent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 1–1.05 equiv, DMF, 25 °C, 1 h),[15] and subsequent in situ anhydride ring-opening with the 6 primary amines **A1–A6** (R^1NH_2, 1 equiv, DMF, 25 °C, 12 h, 64–99%) was conducted on 20 mmol scales to yield approximately 7 g of the monoamides **2** in superb yields (avg = 90%), Table 1. Simply washing the crude product diluted in EtOAc with aqueous acid served to remove unreacted R^1NH_2, EDCI and its reaction byproducts and provided the pure monoamides (≥95% pure). Each monoamide was divided into 10 portions and treated with the 10 primary or secondary amines (**B1–B10**, 1.1 equiv) and PyBOP (1.1 equiv, 2–2.2 equiv *i*-Pr_2NEt, DMF, 25 °C, 16 h, 7–99%) to afford a total of 60 diamides which were purified by sequential acid (10% aqueous HCl), base (saturated aqueous $NaHCO_3$), and saturated aqueous NaCl extractions from EtOAc to remove reagent-derived reaction byproducts, unreacted starting material, R^2NH_2, and PyBOP. Each reaction was conducted on a 1.75 mmol scale corresponding to approximately 600 mg of starting monoamide. The individual yields of the *N*-BOC iminodiacetic diamides **3** ranged from 7–100% (Table 1), with an average of 53%. Irrespective of the reaction

efficiency or product recovery and without deliberate reaction optimization, the purities of the resulting diamides were uniformly satisfactory (≥90–95%) and the identities of products were confirmed by matrix characterization (HRMS, ^{1}H NMR and IR).

Figure 2



The final assembly of the library of 600 compounds entailed 60 coupling reactions of each iminodiacetic acid diamide (**A1B1–A6B10**) with an equimolar mixture of 10 dicarboxylic acids (**C1–C10**) producing 60 sub-libraries of 10 compounds. This was accomplished by acid-catalyzed deprotection of **3** (4 M HCl–dioxane, 25 °C, 2 h, 0.15 mmol) followed by coupling (0.15 mmol PyBrOP, 0.45 mmol *i*-Pr_2NEt, DMF, 25 °C, 12 h, 9–99%) of the crude amine hydrochloride salt with an equimolar mixture of the

Table 1. Isolated Yields (%) of **2** and **3**.

Entry	A1	A2	A3	A4	A5	A6
2	98	99	64	99	99	80
3	A1	A2	A3	A4	A5	A6
B1	90	95	a	75	81	100
B2	12	17	9	18	27	18
B3	81	81	84	67	50	97
B4	89	68	40	56	64	89
B5	97	96	a	68	92	100
B6	57	85	50	43	46	86
B7	13	15	19	13	7	36
B8	43	32	18	26	33	50
B9	20	27	12	16	12	42
B10	99	97	100	72	91	100

[a]Hygroscopic

Table 2. Isolated Amounts (mg) and Yields (%) of the 60 Final Sub-libraries **AXBXC1-10** (**4**).[a]

Entry	A1	A2	A3	A4	A5	A6
B1	49 mg, 99%	49 mg, 99%	48 mg, 99%	49 mg, 99%	50 mg, 99%	36 mg, 74%
B2	10 mg, 24%	13 mg, 29%	37 mg, 84%	10 mg, 21%	14 mg, 32%	6 mg, 13%
B3	36 mg, 79%	46 mg, 93%	20 mg, 41%	4 mg, 9%	26 mg, 56%	38 mg, 59%
B4	36 mg, 74%	39 mg, 76%	22 mg, 46%	37 mg, 73%	28 mg, 57%	36 mg, 73%
B5	47 mg, 99%	50 mg, 99%	49 mg, 99%	50 mg, 99%	31 mg, 59%	42 mg, 82%
B6	18 mg, 44%	58 mg, 66%	9 mg, 21%	22 mg, 50%	12 mg, 27%	29 mg, 66%
B7	11 mg, 29%	28 mg, 66%	18 mg, 46%	21 mg, 50%	11 mg, 26%	14 mg, 34%
B8	7 mg,13%	26 mg, 47%	21 mg, 40%	22 mg, 39%	18 mg, 33%	20 mg, 37%
B9	20 mg, 42%	10 mg, 20%	10 mg, 20%	19 mg, 38%	18 mg, 37%	52 mg, 88%
B10	52 mg, 91%	48 mg, 78%	27 mg, 45%	33 mg, 53%	40 mg, 68%	21 mg, 41%

[a]Yield based on average molecular weight.

dicarboxylic acids **C1–C10** (0.005 mmol each, 0.05 mmol total, 0.67 equiv). The use of the secondary amine in excess for an extended reaction time insured the coupling consumption of the limiting diacid linkers and the near equimolar generation of each compound. Purification by sequential aqueous acid and base extractions removed the unreacted secondary amines, unreacted dicarboxylic acids **C1–C10** as well as any monocarboxylic acid contaminant, the excess reagents (PyBrOP, *i*-Pr_2NEt) and their reaction byproducts. The use of either an acidic ion exchange resin (liquid–solid extraction, DOWEX 50WX8-400)[16] or aqueous 20%

HCl saturated with NaCl improved the mass balance recovery and effectively removed the excess amine reactants and reagents. Using either of these protocols, the final extractive purification provided the individual compounds and the mixture sublibraries at an exceptional level of purity with satisfactory recoveries and the latter was adopted for the preparation of our prototypical library. Using this protocol, each of the 60 sublibraries of 10 compounds was produced in yields ranging from 9–99% (57% average) in amounts ranging from 4–52 mg (Table 2). Irrespective of the conversion, the extractive purification coupled with the scale provided the libraries in high purity (≥90%) and in sufficient quantity suitable for direct broad screening in a variety of assays. Matrix characterization of the 60 sublibraries by MS and ^{1}H NMR confirmed the constitution of the mixtures and a comparison of the sublibrary **A2B6C1–10** with a reconstituted mixture prepared by combining an equimolar amount of the individual components established the integrity of the mixture.

Further extensions of this approach to the preparation of chemical libraries and their use in studying protein and receptor homo- and heterodimerization are in progress and will be disclosed in due course.

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